

## Genetic structure of *Sinularia* (Octocorallia: Alcyoniidae) from Sanya (China) using COI and 28S markers

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### Abstract

In this present study we randomly collected 15 samples of *Sinularia* from Sanya coral reefs which there are belonging to 7 species including *Sinularia grandilobata* (1 specimen), *Sinularia flexibilis* (4 specimens), *Sinularia querciformis* (1 specimen), *Sinularia humilis* (2 specimens), *Sinularia ceramensis* (2 specimens), *Sinularia humesi* (2 specimens), and *Sinularia slieringsi* (3 specimens). Both COI and 28S markers used to study inter-specific genetic variation in *Sinularia*. Based on this, the specific genetic variation analysis within the genus was analyzed. The results showed that the 28S sequence of *Sinularia* is higher than the mitochondrial COI sequence.

**Keywords:** soft coral, genetic variation, phylogeny, species distribution

### Introduction

Soft corals (Cnidaria: Octocorallia) are important structural components of coral reef communities and contributors to coral reef biomass. Most soft corals belong to the family Alcyoniidae, which is abundant and ecologically important members of coral (Mc Fadden *et al.*, 2009; Benayahu *et al.*, 2018, Quattrini *et al.*, 2019) [13, 6, 15]. Despite the abundance and importance of Alcyoniidae, only few ecological studies have been done (Fabricius, 1995, 1997, 1998; Fabricius & Dommissé, 2000; Bastidas *et al.*, 2004; Aratake *et al.*, 2012), partly due to the difficulties in correctly identifying species in the field. Colony morphology is highly variable (Benayahu, 1998), and species classification usually be distinguished by sclerites found within the tissue (Verseveldt, 1980; Benayahu *et al.*, 2018) [19, 6]. Recently, DNA molecular marker techniques have been providing more evident to solve such problems (Lien *et al.*, 2015).

*Sinularia* is a genus of Alcyoniidae with approximately 170-190 described species (van Ofwegen 2002, McFadden *et al.* 2009) [13]. *Sinularia* live in extensive areas of habitats in open water. They also show different size of body (Fabricius and Alderslade 2001) [7]. Verseveldt (1980) [19] classified *Sinularia* into five groups based on morphological pattern of sclerites. McFadden *et al.*, (2009) [13] used molecular methods to divided *Sinularia* in six group, but they were different with the Verseveldt's classification.

Although The South China Sea has many coral reefs with high biodiversity recorded in Taiwan (Benayahu *et al.*, 2018) [6] and Vietnam (Lien *et al.*, 2015) but there is a lack of information about taxonomy of Octocorallia specially *Sinularia* in China coral reefs. Sanya, located in the south coast of Hainan Province in China, including several coral reefs. Therefore, in this study, 15 specimens of *Sinularia* were collected randomly around Sanya. Objectives of the research included: (1) to identify taxonomic status of

collected specimens. (2) to consider phylogenetic relationship among specimens based on mitochondrial fragment of *COI* and nuclear fragment of *28S*. (3) to characterize the difference of molecular variation between mitochondrial *COI* and nuclear *28S*.

### Materials and Methods

#### Study area and sampling

A total of 15 specimens were collected from Sanya city coral reefs (18° 15' 12" N-109° 30' 13" E, South China Sea) (Fig. 1). Taxonomical status were identified according to haplotype patterns of *28S* sequence (McFadden *et al.*, 2009; Benayahu *et al.*, 2018, Quattrini *et al.*, 2019) [13, 6, 15].



Fig 1: Map of *Sinularia* sampling sites in Sanya. © Google Map, 2020.

**DNA extraction, PCR amplification and sequencing**

DNA was extracted from tissue using of corals using DNA Isolation Kit. Two fragments of mitochondrial markers (*COI*) and one nuclear marker (*28S*) were amplified. PCR was carried out on a total volume of 20 µl containing 8 µl of ddH<sub>2</sub>O, 10 µl Taq polymerase, 0.4 µl of DNA solution and 0.8 µl of each primer.

The partially combined fragments of mitochondrial *CO* were amplified using special soft coral primers (McFadden *et al.*, 2004). A fragment of the *28S* nuclear ribosomal gene was amplified using primers suggested by McFadden and van Ofwegen (2013)<sup>[14]</sup>.

**Phylogenetic analyses**

Phylogenetic trees were performed Based on the alignment

of *COI* and *28S* using maximum likelihood (ML) in MEGA-X (Kumar *et al.* 2018). To detect the genetic differentiation of samples a median network was performed using the median-joining algorithm in the Network program ver. 5.0.0.3 (Bandelt *et al.* 1999)<sup>[5]</sup>.

**Results and Discussion**

With regard to our results, collected samples from Sanya belonging to 7 species including *Sinularia grandilobata* (1 specimen), *Sinularia flexibilis* (4 specimens), *Sinularia querciformis* (1 specimen), *Sinularia humilis* (2 specimens), *Sinularia ceramensis* (2 specimens), *Sinularia humesi* (2 specimens) and *Sinularia slieringsi* (3 specimens). Figure 2 and 3 show the photos of studied *Sinularia* and morphology of sclerate for each species



**Fig 2:** Photos of *Sinularia* soft coral in Sanya

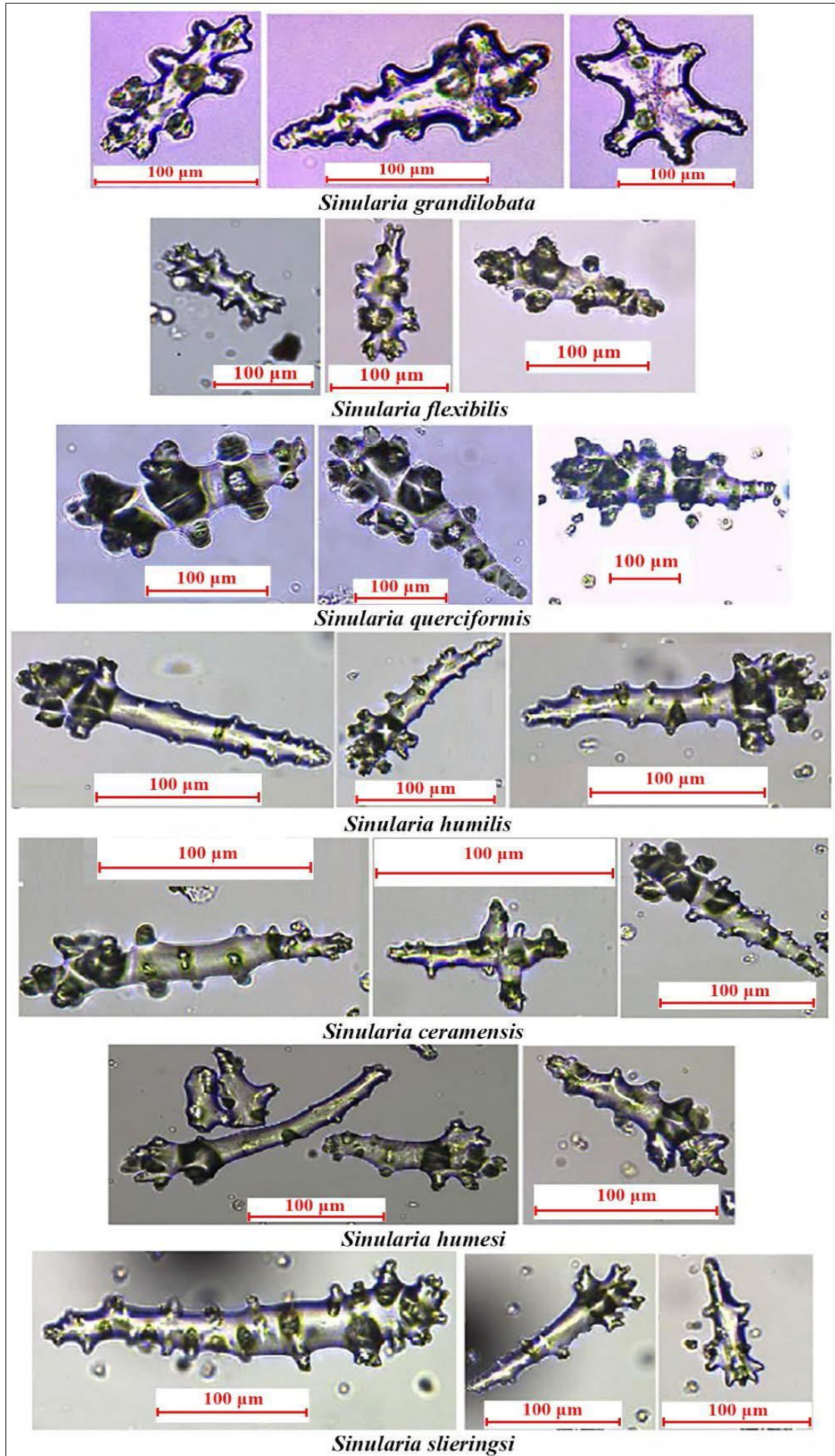
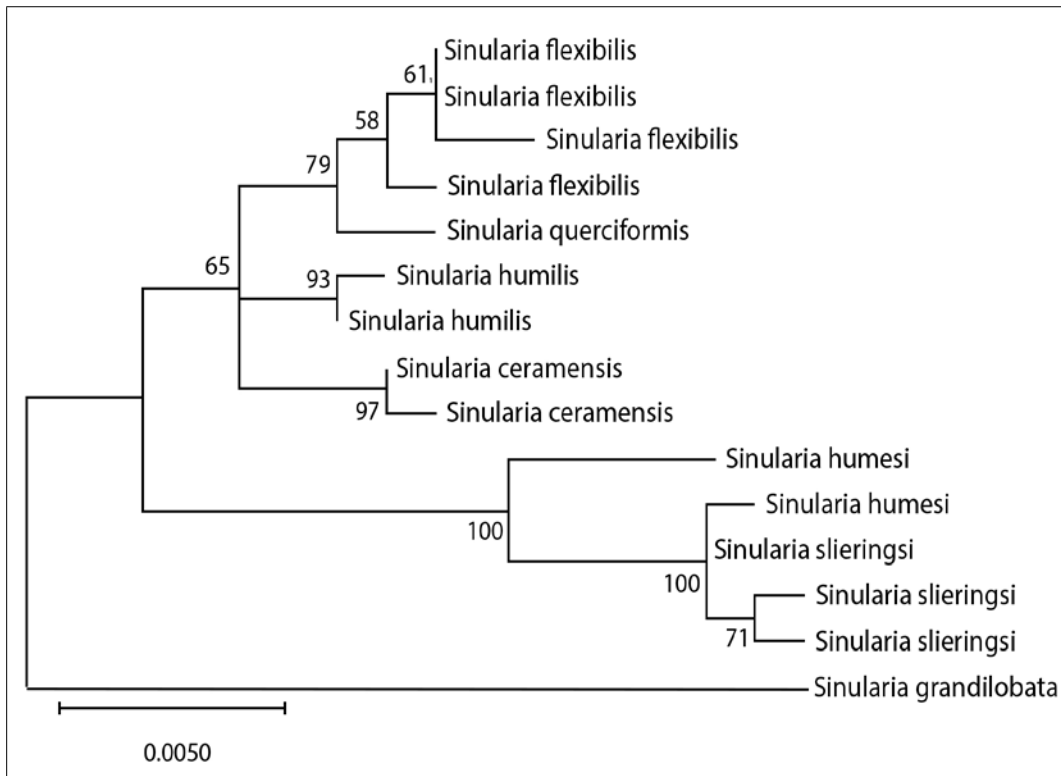


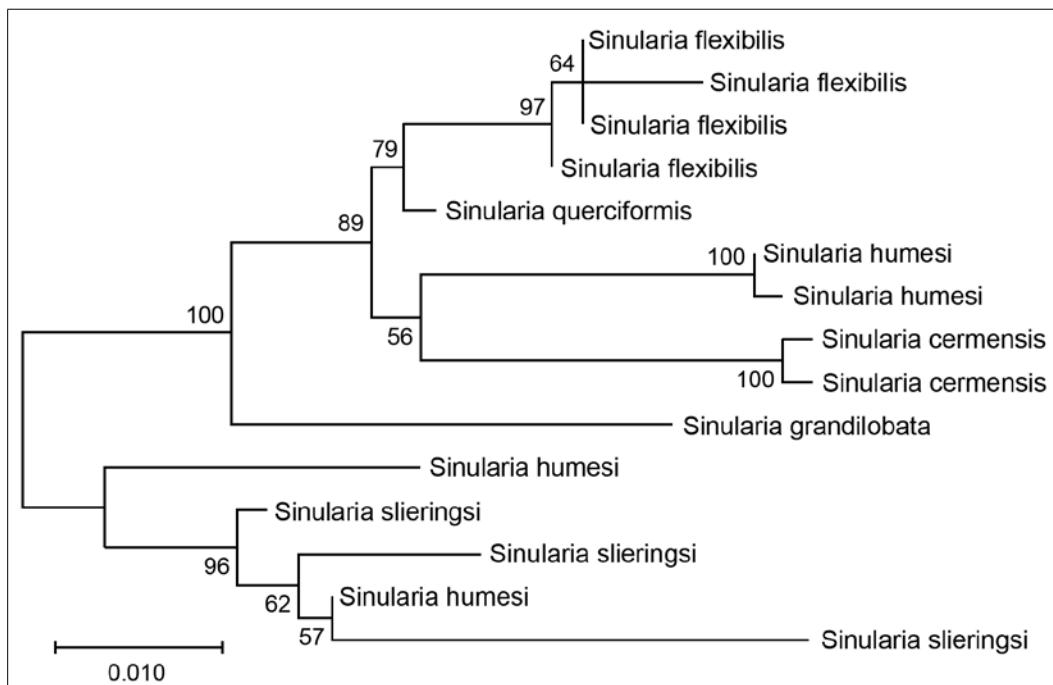
Fig 3: The morphology of spicules of each species.

Phylogenetic tree based on mitochondrial COI marker divided *Sinularia* samples in three major clads that *S. grandilobata* located as a basal clad (Fig. 4). In the other hand, phylogenetic tree using nuclear 28S marker divided

studied samples in two major clads while *S. grandilobata* located as a sister clad for *S. flexibilis*, *S. querciformis*, *S. humilis* (Fig. 5).



**Fig 4:** Phylogenetic tree of *Sinularia* using COI sequences based on the ML approach. The number behind major nodes denotes bootstrap confidential values.



**Fig 5:** Phylogenetic tree of *Sinularia* using 28S sequences based on the ML approach. The number behind major nodes denotes bootstrap confidential values.

Figure 6 showed the COI haplotype distribution network of the *Sinularia* among different species. The 15 COI sequences of *Sinularia* have represented 14 distinct haplotypes, that only two specimens of *S. flexibilis* represented same haplotype (H2) (Fig. 6). Figure 7

displayed the 28S haplotype distribution network of the *Sinularia*. Fifteen sequences have showed 14 separated haplotypes. Same as COI haplotype network, two specimens of *S. flexibilis* have same haplotype (H4). Haplotypes information has been summarized in Table 1. According to

result of COI sequences, *S. grandilobata* showed significant differentiation in genetic structure than other *Simularia*

species and located as basal clad in phylogenetic tree while this species has low variation in 28S gene.

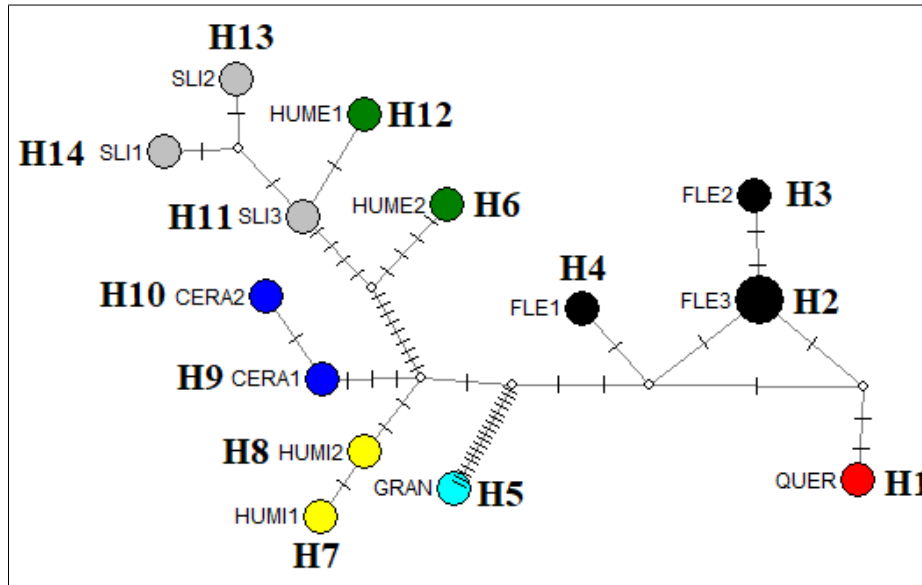


Fig 6: The relationship of COI haplotypes distribution among species.

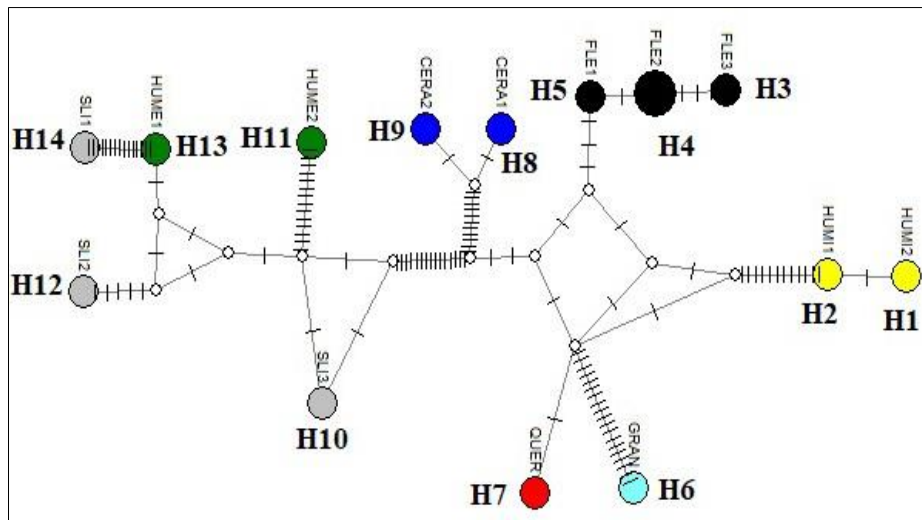


Fig 7: The relationship of 28S haplotypes distribution among species.

Table 1: Haplotypes information for COI and 28S sequences in *Simularia*

Haplotype	COI		28S	
	Species	Individual No.	Species	Individual No.
H1	<i>S. querciformis</i>	1	<i>S. humilis</i>	1
H2	<i>S. flexibilis</i>	2	<i>S. humilis</i>	1
H3	<i>S. flexibilis</i>	1	<i>S. flexibilis</i>	1
H4	<i>S. flexibilis</i>	1	<i>S. flexibilis</i>	2
H5	<i>S. grandilobata</i>	1	<i>S. flexibilis</i>	1
H6	<i>S. humesi</i>	1	<i>S. grandilobata</i>	1
H7	<i>S. humilis</i>	1	<i>S. querciformis</i>	1
H8	<i>S. humilis</i>	1	<i>S. ceramensis</i>	1
H9	<i>S. ceramensis</i>	1	<i>S. ceramensis</i>	1
H10	<i>S. ceramensis</i>	1	<i>S. slieringsi</i>	1
H11	<i>S. slieringsi</i>	1	<i>S. humesi</i>	1
H12	<i>S. humesi</i>	1	<i>S. slieringsi</i>	1
H13	<i>S. slieringsi</i>	1	<i>S. humesi</i>	1
H14	<i>S. slieringsi</i>	1	<i>S. slieringsi</i>	1

In animals, generally mitochondrial markers show 5 to 10

times faster alteration than nuclear genes (Allio *et al.*, 2017) [1]. This trait has caused mitochondrial markers identified as an important evolutionary markers to distinguish species and study the genetic variation of animals (Avise, 1994) [3], also *COI* has showed important results to identify animal species (Smith *et al.*, 2005; Hajibabaei *et al.*, 2006; Tavares and Baker 2008; Baker *et al.*, 2009) [16, 9, 17, 4]. Benayahu *et al.* (2012) documented that *COI* mitochondrial marker cannot identify species of *Klyxum* and *Cladiella*, but nuclear 28S could distinguish species of these genera. Same as our results, Lien *et al.* (2015) also confirmed that different molecular markers show different result for phylogenetic tree. Recently in *Simularia*, sequences of *MutS* and 28S sequences can use to identified species of soft coral (Benayahu *et al.*, 2018; Quattrini *et al.*, 2019) [6, 15]. Based on network distribution, total numbers of mutation have been reported 54 and 89 mutations for *COI* and 28S, respectively. This result documented that 28S nuclear marker has high level of genetic differentiation than *COI* mitochondrial gene.

First comprehensive investigation on soft corals taxonomy has been done by Benayahu *et al.* (2012) from the Penghu Archipelago (Taiwan). They could identify 13 species of *Sinularia* based on morphology of sclerites or corals with identified species from Penghu Archipelago are completely different with species from Sanya. Lien *et al.* (2015) studied on soft coral of Nha Trang bay (Vietnam) from South China Sea. They identified 12 species of genus *Sinularia* using MutS marker which only one species (*S. humilis*) are identical as our identified species from Sanya.

In a recent study, Benayahu *et al.* (2018) [6] have reported 27 species of *Sinularia* from that six species are same with species from Sanya (*S. ceramensis*, *S. flexibilis*, *S. humesi*, *S. humilis*, *S. querciformis*, *S. slieringsi*). Totally it can be conclude that distribution of genus *Sinularia* is completely dissimilar in different localities in South China Sea. It can be attributed to different ecological condition in South China Sea which can effect species distribution.

In conclusion, *Sinularia*, is a common genus of Alcyoniidae in the Indo-Pacific coral reef (van Ofwegen 2002, McFadden *et al.* 2009) [13]. Our results showed that in Sanya among 15 randomly collected samples, *S. flexibilis* has high species density than other species of this genus. Additionally, mitochondrial (COI) and nuclear (28S) markers displayed different position of species on phylogenetic trees which it can be referred to disconnected evolutionary history for nuclear and mitochondrion genomes.

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